Protein analysis of *Bifidobacterium longum* ATCC 15708 grown on different prebiotics

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1. ABSTRACT

Bifidobacterium spp. are the major beneficial bacteria found in human intestinal tract. They play important roles in human nutrition and health. *B. longum* is one of the predominant species, especially in infants. It is well documented that prebiotics, such as fructooligosaccharide (FOS), galactooligosaccharide (GOS), and inulin, stimulate the growth of *Bifidobacterium*. However, this stimulatory effect has not been examined at transcriptional or translational level. In this study, we compared the proteomes of *B. longum* ATCC 15708 grown on FOS, GOS, and inulin, with glucose serving as a control substrate. Cell-associated proteins were prepared using sarcosyl treatment and analyzed by 2D electrophoresis. The proteomic profiles were then compared using the Phoretix 2D software. From the cultures grown on glucose, FOS, GOS, and inulin, 39, 46, 55, and 79 cell surface proteins were detected, respectively. Thus, more cell-surface proteins were expressed when this strain was grown on prebiotics than grown on glucose. The FOS-, GOS-, and inulin-grown cultures each shared 26-29 proteins spots with the glucose-grown cultures. The rest of the proteins may be specifically expressed for uptake and/or extracellular hydrolysis of the prebiotics. Additionally, several proteins appeared to be highly expressed when grown on these prebiotics than on glucose. Apparently, *B. longum* needs to express different genes or operons for the uptake and/or extracellular hydrolysis of different prebiotics. Identification of these differentially expressed proteins will expand our understanding of the molecular mechanism of the prebiotic effect on *B. longum*.

2. INTRODUCTION

- ***Bifidobacterium longum* has an important role in a balanced intestinal flora.**
  - ***B. longum* produces lactic acid and acetic acid that inhibit the growth of some pathogenic bacteria**
  - ***B. longum* may modulate immune response in the gut and may have anti-carcinogenic activity**
- **Prebiotics that can be beneficial to host health.**
  - **Prebiotics is present in human milk, but some can be often extracted from plant sources.**
  - **Prebiotics can help stimulate the growth of beneficial bacteria such as *B. longum*.**

2. INTRODUCTION (continued)

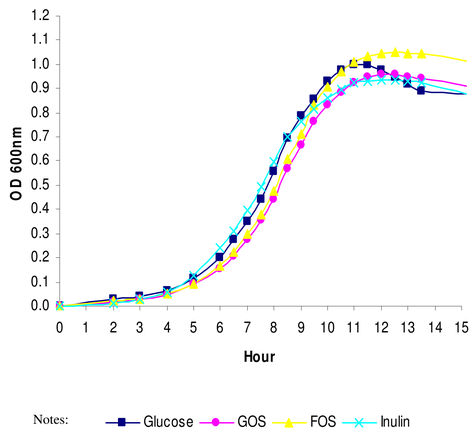
- **Proteomic approach may help reveal the genetic and biochemical basis of prebiotic fermentation by *B. longum*.**
  - **Recently, a theoretical proteome reference map of *B. longum* NCC2705 has been generated.**
  - **Different changes in protein expression between glucose- and prebiotics-grown *B. longum* can be examined by proteomic analysis.**
  - **Identification of these differentially expressed proteins will advance our understanding of the molecular mechanism underpinning the effect of prebiotics on *B. longum*.**

3. EXPERIMENTAL

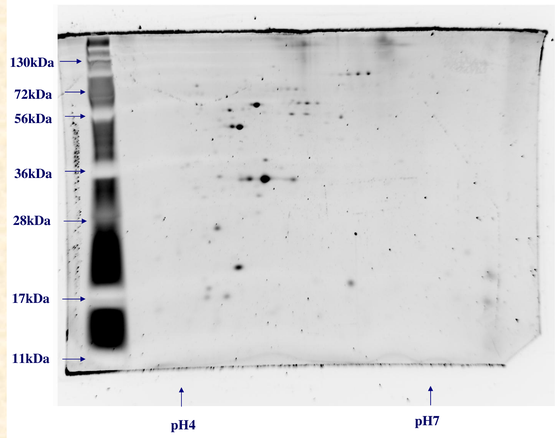
- **Bacterial strain: *B. longum* ATCC 15708**
- **Growth on GOS, FOS, and inulin with glucose serving as a control substrate in a basal medium.**
- **Determination of growth rates.**
- **Extraction of cell surface proteins.**
- **2D PAGE analysis.**
- **Proteomic profile analysis by Phoretix 2D software.**

4. RESULTS

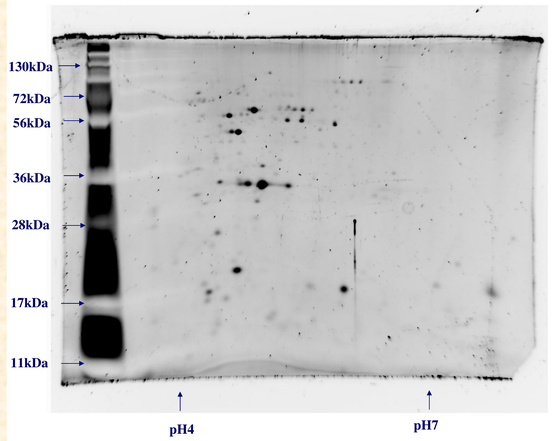
- **Growth of *B. longum* in the basal medium with 1% glucose, GOS, FOS, or inulin as the sole carbon source.**



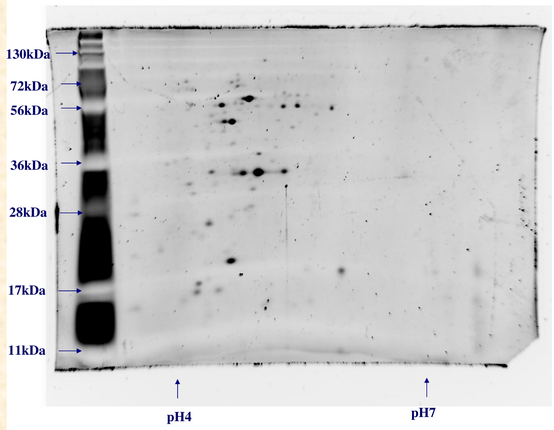
- **2D PAGE analysis of cell surface proteins.**
  - **2D gel of the cell surface proteins in a glucose-grown *B. longum* culture.**



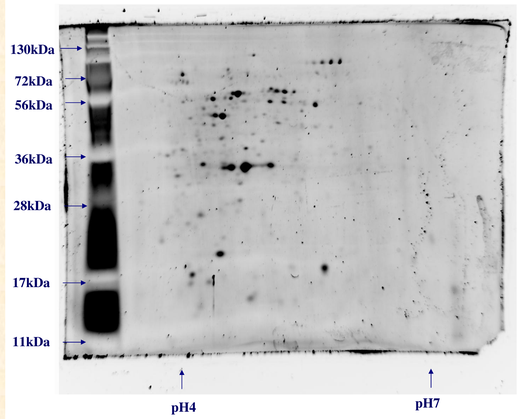
  - **2D gel of the cell surface proteins in a GOS-grown *B. longum* culture.**



  - **2D gel of the cell surface proteins in a FOS-grown *B. longum* culture.**



  - **2D gel of the cell surface proteins in an inulin-grown *B. longum* culture.**



5. SUMMARY

- **There is no significant difference in growth rate of *B. longum* among the tested substrates.**
- **More cell-surface proteins were expressed when *B. longum* was grown on prebiotics, specially on inulin, than grown on glucose.**
- **Several proteins appeared to be highly expressed when grown on these prebiotics than on glucose.**
- **These proteins may be specifically expressed for uptake and/or extracellular hydrolysis of the prebiotics.**
- **Proteins differentially expressed when grown on prebiotics are being identified.**